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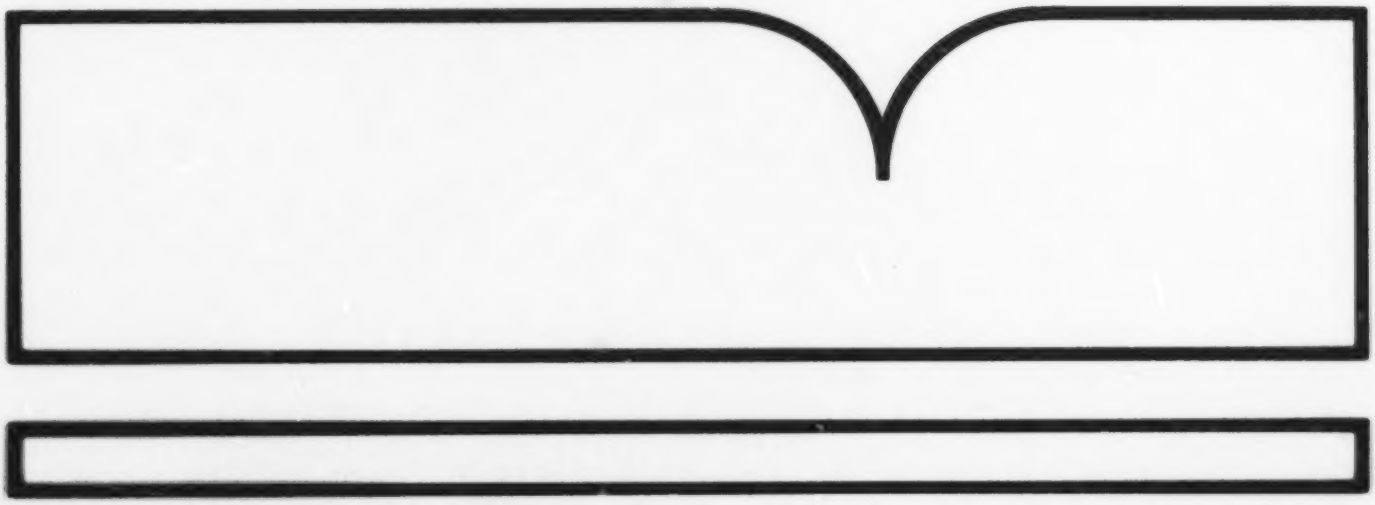

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Mutagenicity of Combustion Emissions from a Biomedical Waste Incinerator

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The Ames Salmonella typhimurium (TA98) assay was used to determine the mutagenicity of stack fly ash from a medical/pathological waste incinerator. Stack fly ash was also collected from a boiler plant adjacent to the incinerator and ambient air particles (upwind and downwind of the incinerator and boiler facilities) were collected and bioassayed. Downwind particulate mutagenicity (revertants per cubic meter of air) was significantly greater than upwind particulate mutagenicity. Mutagenic emission rate estimates (revertants per kilogram waste feed) for the incinerator and boiler were less than estimates published for wood stoves, automobile gasoline engines, and residential furnaces. Incinerator stack fly ash and downwind ambient air particulate samples collected during incinerator auxiliary burner failure demonstrated significant increases in mutagenicity compared to samples collected during routine incinerator operation.

Introduction

Incomplete combustion products from various sources contain polycyclic organic compounds; in several cases these products have been reported as human carcinogens (1,2). Mutagenicity is frequently used in screening assessments for genotoxic compounds which may be carcinogenic and to which humans are exposed. Complex environmental mixtures have been monitored using short-term mutagenicity assays. The relative mutagenic and carcinogenic activities of several combustion emissions have been characterized and compared. These include emissions from residential wood stoves and oil heaters (3,4), utility power plants (5), municipal incinerators (6), diesel and gasoline vehicles (7), and fluidized-bed coal combustion units (8).

Incineration has been suggested as an effective means of destroying hospital and biomedical research waste (9). There are indications that unconventional contaminants are emitted from combustion of plastic-rich mixtures such as those that hospitals and biomedical research facilities generate. Combustion of polyethylene, polypropylene, polyvinyl chloride and plastic-rich waste has indicated that in addition to particles and carbon monoxide (CO), other substances such as ethane, propane, propylene, hydrochloric acid, furans, and dioxins are emitted (10, 11). Federal guidelines controlling such incinerators are not well-defined and state regulations vary significantly (9, 12).

Various strategies have been used to measure the contribution of mutagenic combustion sources to total ambient aerosol mutagenicity, including source-oriented modeling and source emission potencies (i.e., the biological response per unit mass of extractable organic matter) and source appropriation based upon ambient measurements of mutagenicity and associated "marker" compounds for suspected sources of the mutagens. The source-oriented approach is advantageous for directly describing mutagenic potency emission rates for comparing various sources; however, mass emission rate, dispersion modeling, atmospheric transformations of mutagens, meteorological data, and receptor demographics must be characterized for conducting comprehensive exposure assessments. In contrast, although receptor-oriented methods better estimate potential exposure conditions, combustion conditions associated with increases in mutagenic emission rates are not definable. This investigation

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used both source sampling and receptor sampling strategies to characterize the contribution of a biomedical waste incinerator and an industrial boiler to ambient air mutagenicity.

Collection of Samples

The most readily available sampler for receptor site sampling is the standard Total Suspended Particulate (TSP) high-volume sampler (General Metals, Cleveland, OH). These samplers were used to collect "upwind" and "downwind" (relative to the incinerator and boiler facilities) ambient air particles at the National Institutes of Health (Bethesda, MD). Samples were collected for 24 hour periods, and the sampling rate was determined by use of a rotometer. Samples were collected on Whatman (Maidstone, England) 20 x 28 cm high purity fiberglass filters. Figure 1 shows the location of the hi-vol samplers (A-D) as well as the location of the incinerator (#3) and the boiler (#1) from which stack fly ash samples were taken. Because of the location of the incinerators with respect to the boilers, the ambient air downwind of these sources included particulate emissions from all sources (i.e., the four boilers, two of which operate 24 h per day, seven days per week and the three incinerator stacks, two of which operate from 7:30 am to 7:00 pm, Monday through Friday, and are located within 15 to 30 m from the boiler stacks).

Concurrent with the ambient air sampling for this study, the Radiation Safety Branch (RSB), Division of Safety, NIH conducted ambient air sampling (both particles and gases) for $Iodine_{125}$ and $Iodine_{131}$ originating from the incinerators. Both radioisotopes were detected on the particulate and charcoal filters of low-volume samplers located at the sampling sites where the high-volume samplers were being used. The detection of these isotopes suggested that incinerator emissions were being dispersed to the sampling sites used to collect ambient particulate samples for mutagenicity testing. However, the percentage of sample collected in the high-volume samplers attributable to the incinerator emissions was not determinable.

Integral to the ambient air sampling program were daily records of meteorological parameters (e.g., wind direction, temperature, and humidity). Samples were designated as "upwind" or "downwind" if they were collected when the prevailing wind was in the NW-SE vector (see Figure 1) or quadrant for more than 50% (approximately 12 hours) of the total sampling period (approximately 24 hours). Samples were collected between July and November 1987. During this period, the incinerators were burning medical/pathological waste and the boilers were burning Number 6 residual fuel oil. All three incinerators are controlled air units.

In addition to the ambient air particulate samples, incinerator and boiler stack fly ash samples were collected using a modified high-volume sampler (General Metals, Cleveland, OH) (Figure 2) using fiberglass filters. The sampling probe of the unit was modified to permit immersion in wet ice as well as to provide for the introduction of filtered ambient air. The purpose of the probe extension and dilution air port was to decrease the temperature of the sampled stack gases (1,120 F°) to a temperature of approximately 100 F°. The lower temperature is thought to contribute to the condensation of organics onto the surface of the particles prior to collection on the filter. Temperature inside the sampling probe upstream of the filter medium was continuously monitored using a temperature probe and remote meter readout (YSI model 42SF Tele-thermometer; 40 F° to 300 F°). Stack samples were collected at a rate in the range of 35 - 50 cubic feet per minute (cfm). The incinerator had a flow rate range of 5,361 - 5,506 cfm. The sampling velocity range of the hi-vol sampling instrument was determined to be 11.9 - 17.0 ft/s versus the NIH incinerator #3 exit velocity range of 18.2 to 18.7 ft/s. Thus, sampling isokinetically did not occur and could have resulted in skewing the distribution of particles sampled. In this situation, the particle concentration may have been overestimated (13). However, because the sampling probe of the hi-volume stack sampler was not aligned with the stack gas stream lines, the particle concentration may be underestimated (13). Thus, these two competing anisokinetic factors existed during incinerator stack sampling. The primary objective of the hi-volume stack sampler was to obtain sufficient particulate sample mass (at an appropriate temperature range) to conduct chemical and biological testing. Incinerator stack samples were collected on six different days during the months of October and November 1987. Sampling times ranged from 20 to 60 min. During sampling the incinerator was being "fine-tuned" to determine optimal combustion parameters. Thus, some samples collected during "routine" incinerator operation may actually represent particulate emissions during sub-optimal combustion conditions. Samples were also collected during an auxiliary burner failure on incinerator #3. These samples represent particulate emissions during "upset conditions". Waste feed rate (lbs/hr), incinerator temperature (both primary and secondary chambers), as well as stack temperature were recorded during each sampling interval.

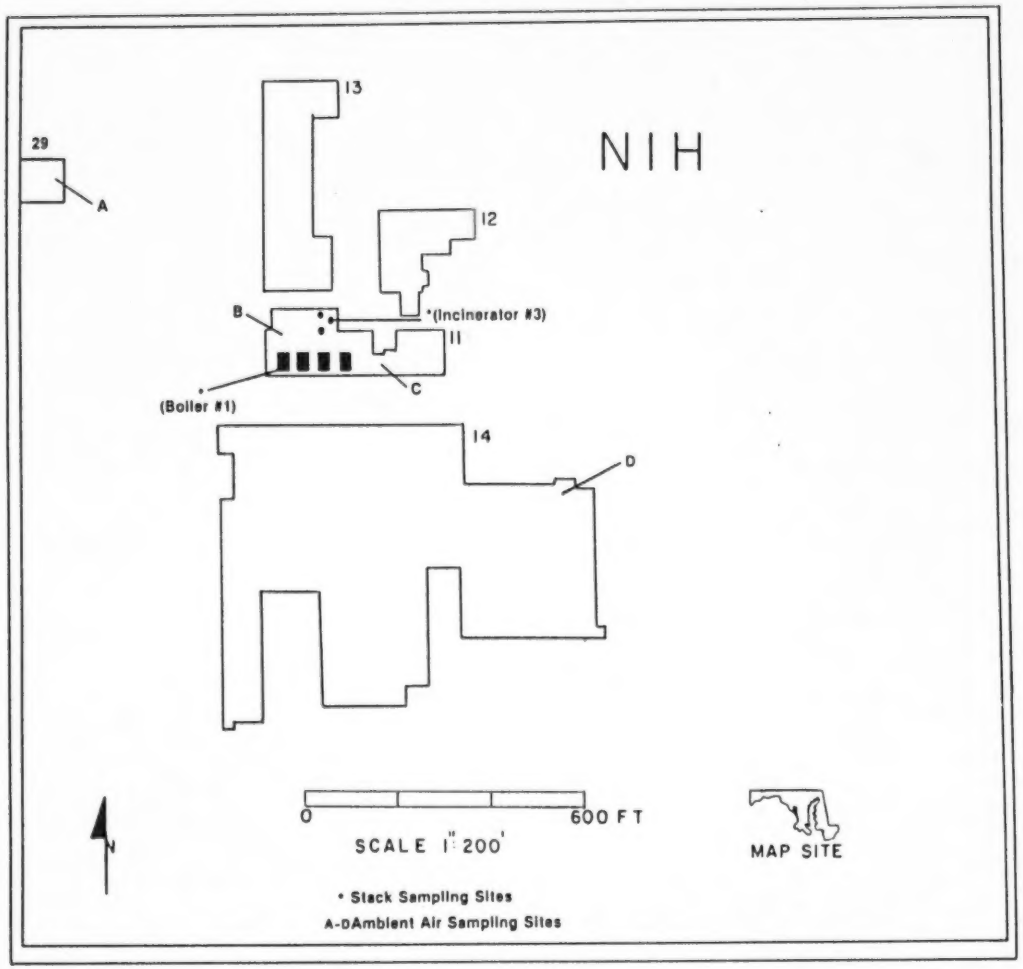

Figure 1. National Institutes of Health, Bethesda, MD; sampling sites.

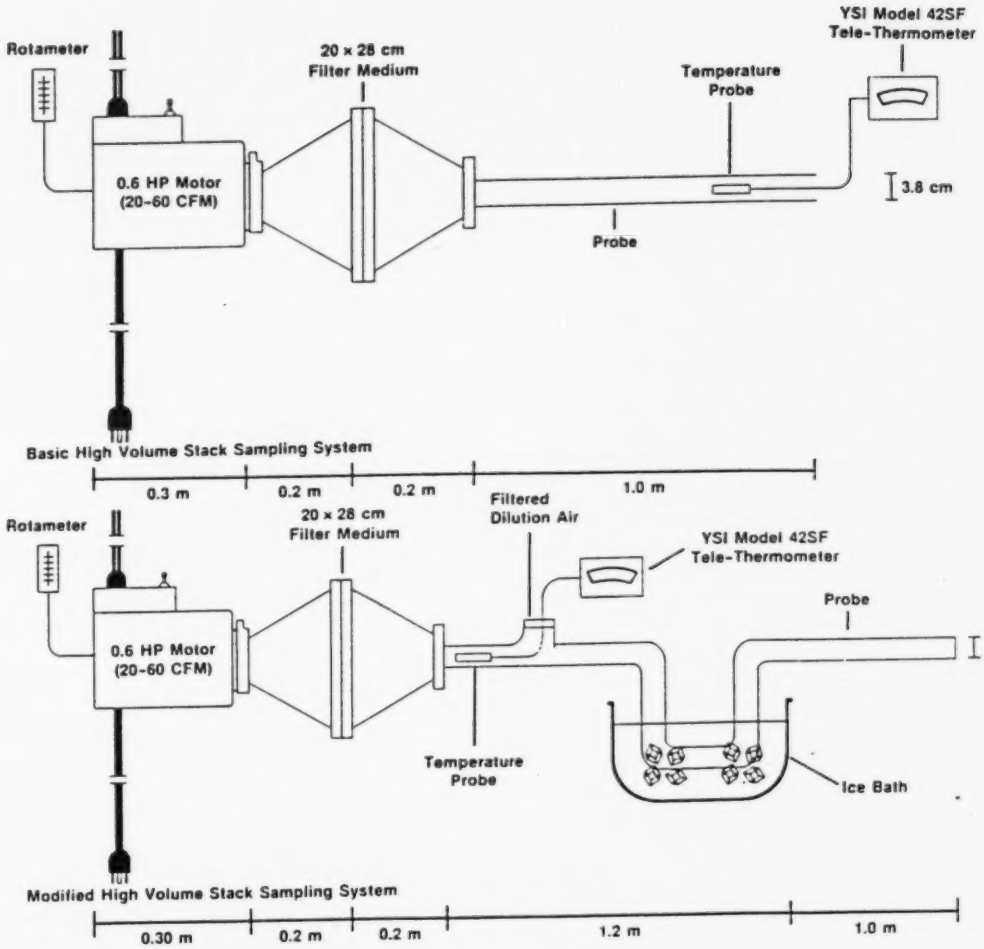

Figure 2. High volume stack sampling systems.

Boiler plant emissions were also collected employing the method used for the incinerator fly ash collection. However, the sampler did not have the 2.4 m sampling probe (see Figure 2) for dilution air intake and ice bath cooling. The boiler stack temperature was approximately 400 F°. Samples were collected for 40 - 60 min. Fuel feed rate (gallons/day) was determined for each sampling day. The cubic meters of air sampled, Total Suspended Particulate (TSP), and mass collected were determined for all filter samples.

Sample Transportation and Storage

Immediately after completion of sampling, all filter samples were folded, double-bagged in labeled plastic freezer bags, and placed on dry ice pending transport to the weighing room on the NIH campus. After weighing, the samples were transported to the Frederick Cancer Research Facility, National Cancer Institute, Frederick, MD. To minimize sample degradation or reaction, filters were stored in the dark at -80 C°. Frozen particulate samples were stored for a maximum of 20 days prior to chemical extraction and bioassay.

Determination of Total Suspended Particulate (TSP)

As described by the standard EPA method (15) the weight (μg) of particles captured on the filter (used to calculate TSP) is determined by obtaining the weight difference between desiccated, pre-weighed filters and the filters after sampling. Furthermore, filters must be desiccated after sample collection to establish the same temperature and humidity conditions as the pre-weight. However, this standard method is not acceptable if the primary objective is to prevent degradation and reaction of the organics for subsequent bioassay and chemical analyses.

Thus, a modified weighing method was used for this study to allow the determination of TSP while maximizing the preservation of organics associated with filter samples. The modified method involved use of a controlled weighing room that maintained a temperature of approximately 20 C° and a relative humidity of approximately 25%. Filters to be used for sampling were desiccated for 24 h, then removed from the desiccator and allowed to equilibrate to room temperature and humidity for a 10 min period prior to determining their tare weight. After filters were used for collecting particulate samples, the filters were transported to the weighing room, removed from the dry ice container and allowed to equilibrate to room temperature and humidity conditions for a 10 min period before weighing on an analytical balance (Mettler). The filters were then immediately resealed in plastic bags and placed on dry ice for transport to the laboratory. The magnitude of the effect of the modified weighing method on TSP determination was tested by comparing filter weights using the modified method and the EPA method (15). The result of this comparison (paired t-test) revealed a difference between the weighing methods of 0.073% ($p = 0.0169$).

Sample Preparation and Bioassay

All ambient air samples collected on the same days as the incinerator and boiler samples were extracted and bioassayed at the same time. Ambient air samples collected on days when the incinerator and boiler samples were not collected were extracted and bioassayed in weekly groups. All samples were ultrasonically extracted in dichloromethane (DCM) using an 85 watt sonicator bath (Ultramet II Sonic Cleaner, Buehler Ltd, Evaston, IL) for 10 min at 25 C°. The DCM extract was then filtered through a 0.45 μm Teflon™-coated filter (Millipore Corp., Bedford, MA) into a round-bottom flask (500 ml). The sonication and filtration procedure was repeated two more times using 110 ml of DCM with each repetition. The extraction solvent for each sample was rotary-evaporated at 40 C° using individual 500 ml round-bottom flasks to approximately 2-3 ml. Each sample was then quantitatively transferred to a 10 ml volumetric flask. Portions of the extract not immediately used for preparation of gravimetric or bioassay samples were stored at -20 C°.

A gravimetric determination of the extractable organic matter (EOM) was performed by placing 0.2 ml aliquots in tared aluminum weighing pans (duplicates for each sample) and allowing the solvent to evaporate in a fume hood, followed by desiccation for 24 h before being reweighed. In addition, 0.5 ml aliquots were placed in a gas chromatograph vial and stored at -20 C° for subsequent gas chromatography (GC) analysis.

The EOM was solvent exchanged into dimethyl sulfoxide and assayed by previously published procedures (16, 17) with the modifications of Andrews et al. (18), using Salmonella typhimurium TA98

both with and without metabolic activation (MA). Positive control chemicals used included 2-nitrofluorene (-MA) at a concentration of 3.0 μg/plate and 2-aminoanthracene (+MA) at a concentration of 0.5 μg/plate. Blank filters which were extracted and bioassayed demonstrated no significant mutagenic activity. Samples were tested at 5-7 concentrations (e.g., ranging from 10 to 1000 μg of extractable organic matter per plate).

Results and Conclusions

Linear regression (least-squares) analyses were performed for all dose-response data using the model of Bernstein et al. (19). Slope values (directly related to mutagenic potency) were expressed as revertant colonies per μg of extractable organic matter or as revertant colonies per m^3 of air sampled, as derived from the linear portion of the dose-response curve. Mutagenic potency comparative analyses were performed using the slope values obtained from the regression analysis. The comparative methods used were the t-test and the Wilcoxon Rank Sum Test (20). Additional methods were developed to compare dose-response data which behaved as a non-linear function (21).

Table 1 contains a comparison matrix for the mean mutagenic potencies of upwind and downwind ambient air samples and incinerator and boiler stack samples collected during "routine" operation conditions. Mutagenic potency expressed as revertants per cubic meter of air sampled was significantly different for upwind and downwind samples. However mutagenic potencies were not different for the incinerator and boiler samples. This observation is interesting given the dramatic differences in the composition of medical/pathological waste and grade 6 residual fuel oil. However, the combustion temperatures were similar for both the incinerator (899 C° in the primary chamber and 1,093 C° in the secondary chamber) and boiler (899 C° in the fire box) as well as the particulate collection temperatures (43-77 C° for the incinerator and 29-104 C° for the boiler).

Figure 3 illustrates the mutagenic potencies (revertants/m^3 with and without metabolic activation) of stack samples as well as upwind and downwind samples collected during "upset" combustion conditions due to incinerator auxiliary burner failure. Stack sample #1 (45 min) was collected immediately following burner failure, whereas, stack sample #2 (45 min) was collected 1 hour after burner failure. The ambient air particulate samples (24 hr) were collected on the day that burner failure occurred. Significantly increased mutagenic potencies were observed for the stack fly ash extracts as well as the downwind particulate extract. Chromatographic analyses of these samples revealed dramatic increases in the total chromatographable organics during "upset" combustion conditions suggesting that the increases in mutagenicity were related to changes in organic emissions during burner failure.

Using the least-squares slopes for samples obtained from the incinerator and boiler, as well as the waste (or fuel) feed rate (e.g., kg/h) and the particulate (mg/h) and organic emission rate (mg/operating day), mutagenic emission rates were estimated. Mutagenic emission rates are expressed as "revertant equivalents" per kg waste (or fuel) feed and "revertant equivalents" per operating day. Table 2 lists the comparison of mutagenic emissions rates for various combustion sources including the incinerator and boiler sampled in this study. The results obtained in this study show that the incinerator and boiler mutagenic emission rates are significantly lower than the rate estimates for a wood stove. However, it must be recognized that the sampling conditions used in this study differed from those used in assessing other combustion source emissions.

This study suggests that mutagenicity may be a useful surrogate indicator of combustion efficiency and may also be applicable to receptor modeling in complex airsheds. Research should be pursued concerning these applications and their potential associations with conventional indicators of combustion such as residence time, temperature, destruction and removal efficiency, total unburnt hydrocarbons and CO_2 and CO concentrations. Future research should include isokinetic sampling, continuous monitoring of combustion performance parameters, and bioassay-directed fractionation of sample extracts. Furthermore, the use of additional short-term assays should be encouraged to relate combustion parameters and emissions to endpoints of toxicological significance.

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Table 1. Comparison of mutagenic potency: revertants per cubic meter of air.

I. Revertants per cubic meter of air without metabolic activation (mean +/- standard error):

Upwind (UW)	(N=7):	2.40 +/- 0.67
Downwind (DW)	(N=10):	10.94 +/- 1.22
Incinerator (INCIN)	(N=18):	70.92 +/- 21.90
Boiler (BOIL)	(N=8):	54.39 +/- 21.56

Probability (p) Matrix:

	DW	INCIN	BOIL
UW	0.00005[a] 0.0001[b] 0.0008[c]	0.0061[b] 0.0002[c]	0.047[b] 0.0015[c]
DW		0.014[b] 0.0017[c]	0.084[b] 0.030[c]
INCIN			0.65[b] 0.64[c]

II. Revertants per cubic meter of air with metabolic activation (mean +/- standard error):

Upwind (UW)	(N=7):	2.19 +/- 0.64
Downwind (DW)	(N=10):	8.00 +/- 1.16
Incinerator (INCIN)	(N=15):	30.16 +/- 5.64
Boiler (BOIL)	(N=8):	28.32 +/- 9.73

Probability (p) Matrix:

	DW	INCIN	BOIL
UW	0.0008[a] 0.0015[b] 0.0054[c]	0.0002[b] 0.0002[c]	0.031[b] 0.0032[c]
DW		0.0015[b] 0.0003[c]	0.076[b] 0.046[c]
INCIN			0.86[b] 0.50[c]

[a]One-sided t-test.
[b]Two-sided t-test.
[c]Wilcoxon Rank Sum test.

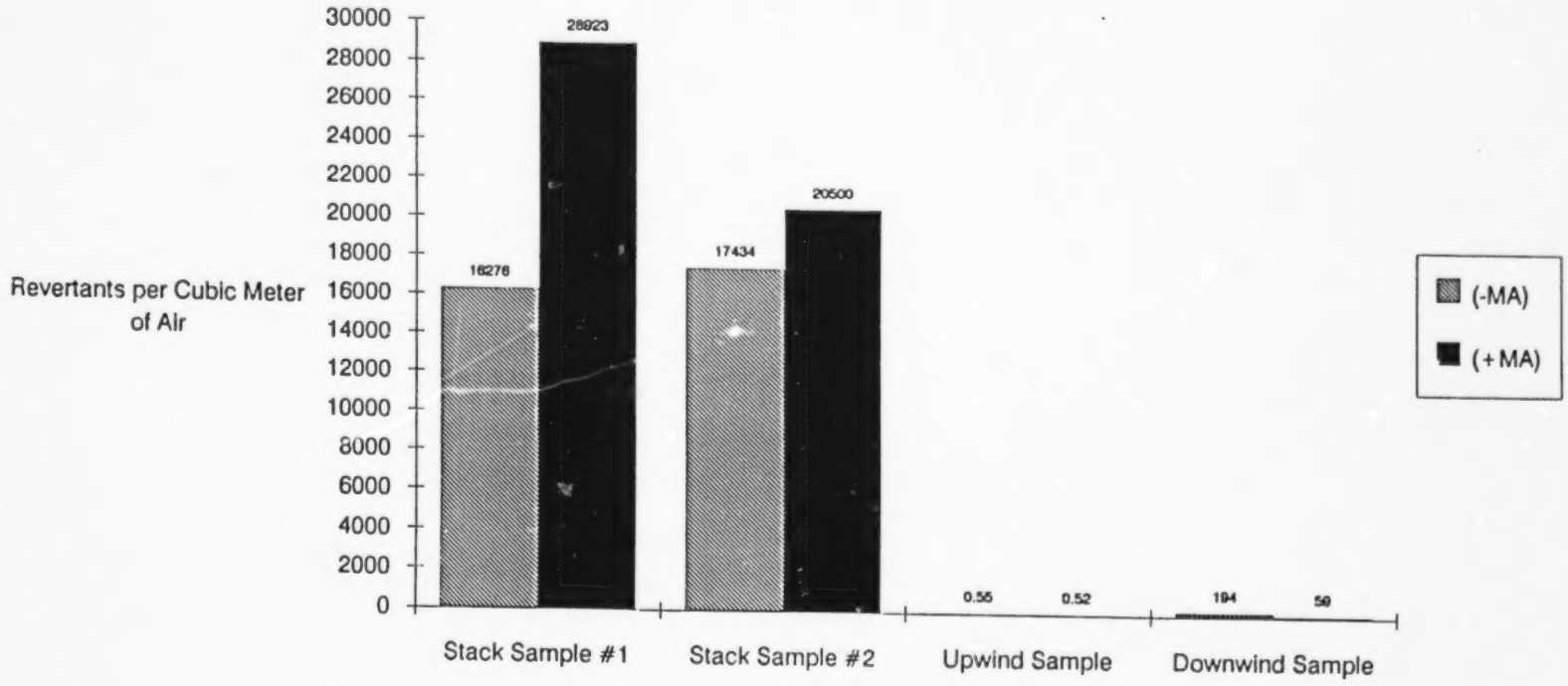
Table 2. Comparison of mutagenic emission rates for various combustion sources.

Source	Fuel type	Mutagenic potency[a] (revertants/μg)	Organic emission rate[b] (mg/kg)	Mutagenic emission rate[c] (revertants/kg)
Incinerator	Medical/ Pathological Waste	0.5 ± 0.2[d] (0.6 ± 0.2[e])	21.5 ± 4.6[d]	4,100 ± 895[d] (10,000 ± 2,915[e])
Industrial boiler	No. 6 fuel oil	0.4 ± 0.1[d] (0.7 ± 0.3[e])	6.1 ± 0.7[d]	2,226 ± 726[d] (4,195 ± 1,566[e])
Gasoline Engine				
Mustang II	Leaded gasoline	3.5	43	140,000
Residential Heaters				
Woodstoves	Pine	1.3	8940	12,000,000
	Oak	0.9	3096	2,800,000
Residential oil				
Furnace #1	No. 2 fuel oil	2.0	21	40,000
Furnace #2	No. 2 fuel oil	5.1	70	360,000

[a]*Salmonella typhimurium* TA98 with metabolic activation.
[b]Organic (mg) emitted per kg of fuel combusted.
[c]Revertants per kg of fuel combusted.
[d]With metabolic activation; determined from dissertation research; mean value ± standard error.
[e]Without metabolic activation; determined from dissertation research; mean value ± standard error.

Adapted from Lewtas (1,2)

Figure 3. Mutagenic potencies of particulate samples collected during incinerator #3 auxiliary burner failure: revertants per cubic meter of air.



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